Whole Cell Panning of Libraries by
Magnetically–Activated Cell Sorting (MACS)

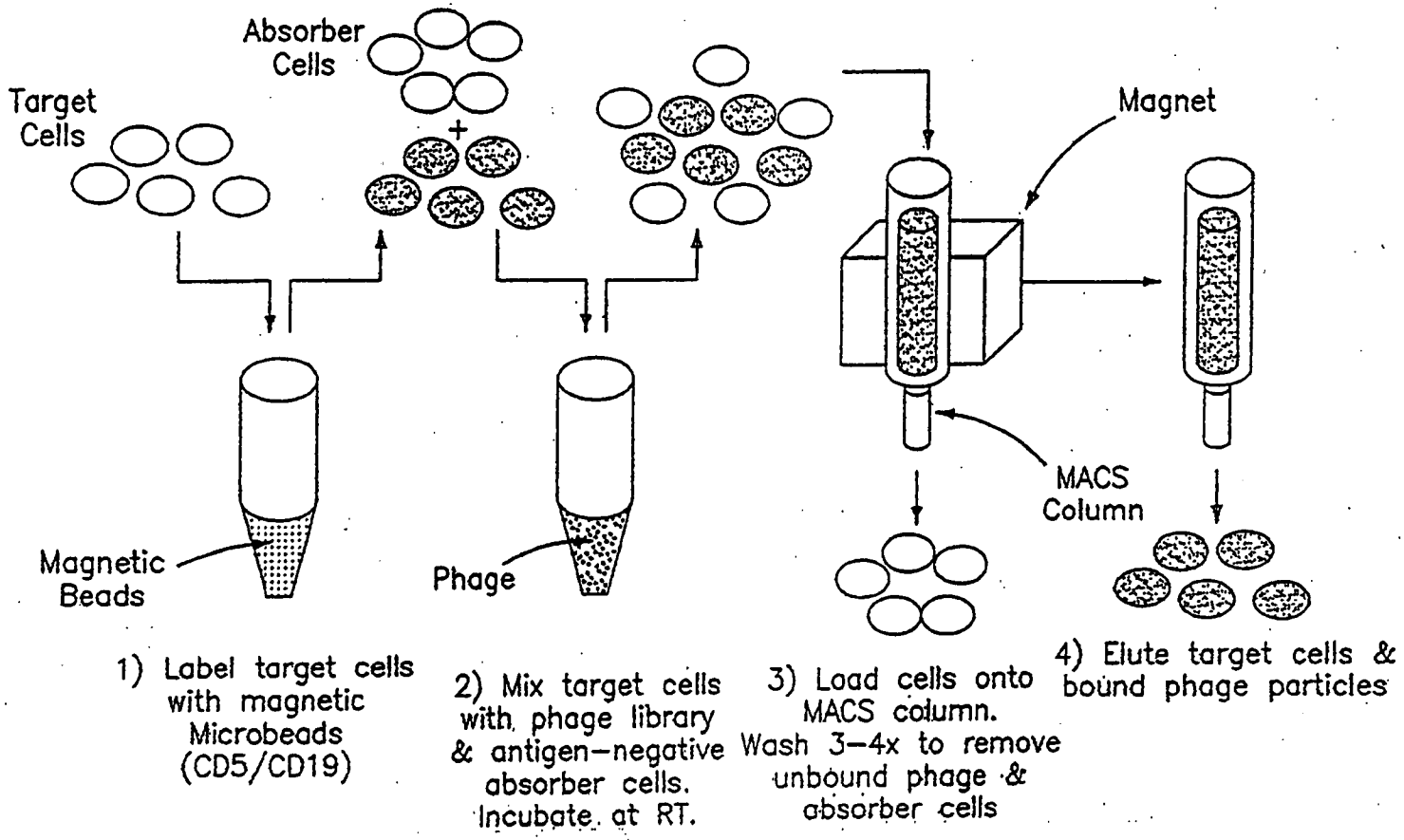

Fig. 1

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Fig 2. CELL ELISA-5/5/00

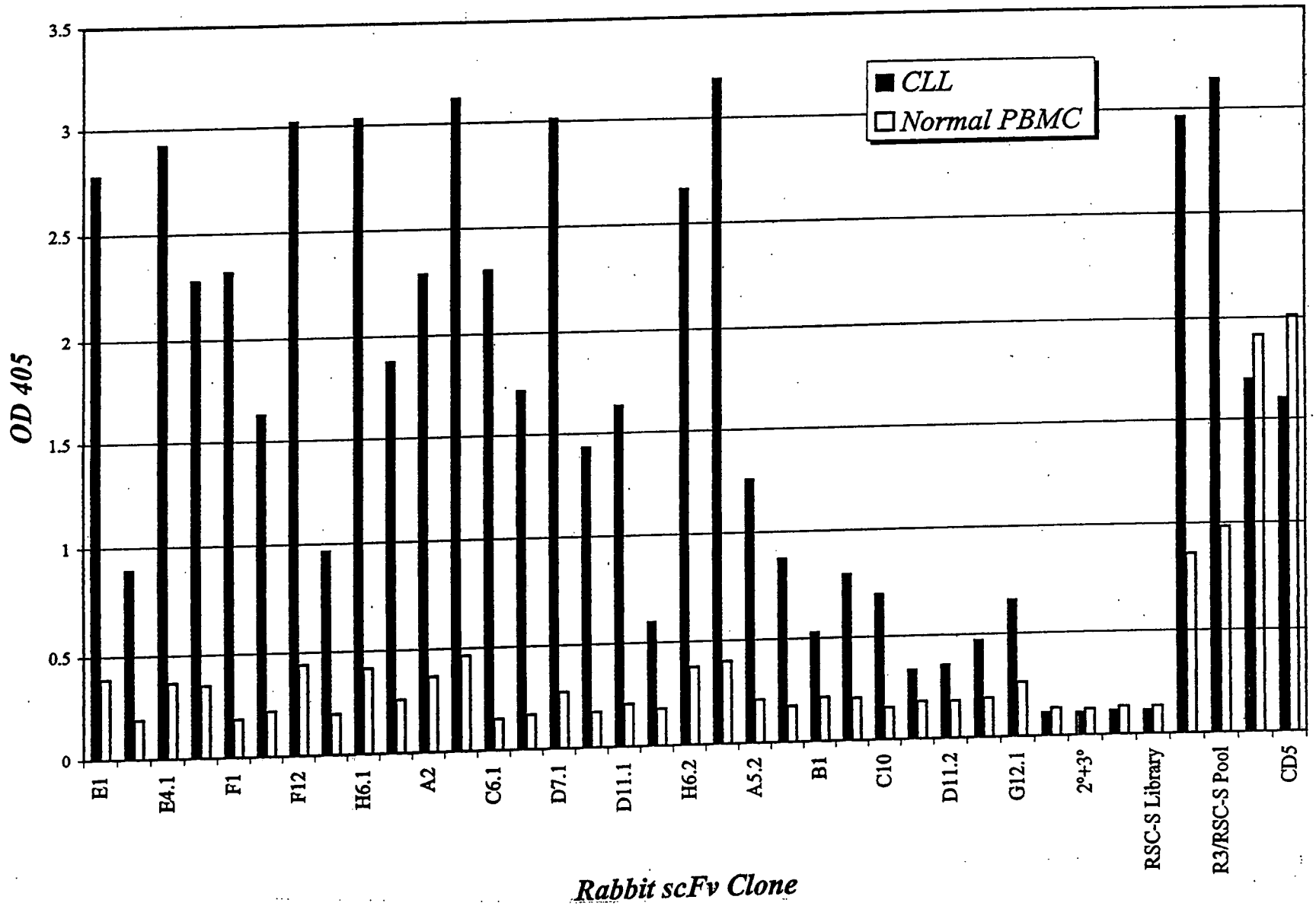

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Fig 3a. CELL ELISA 4/22/00

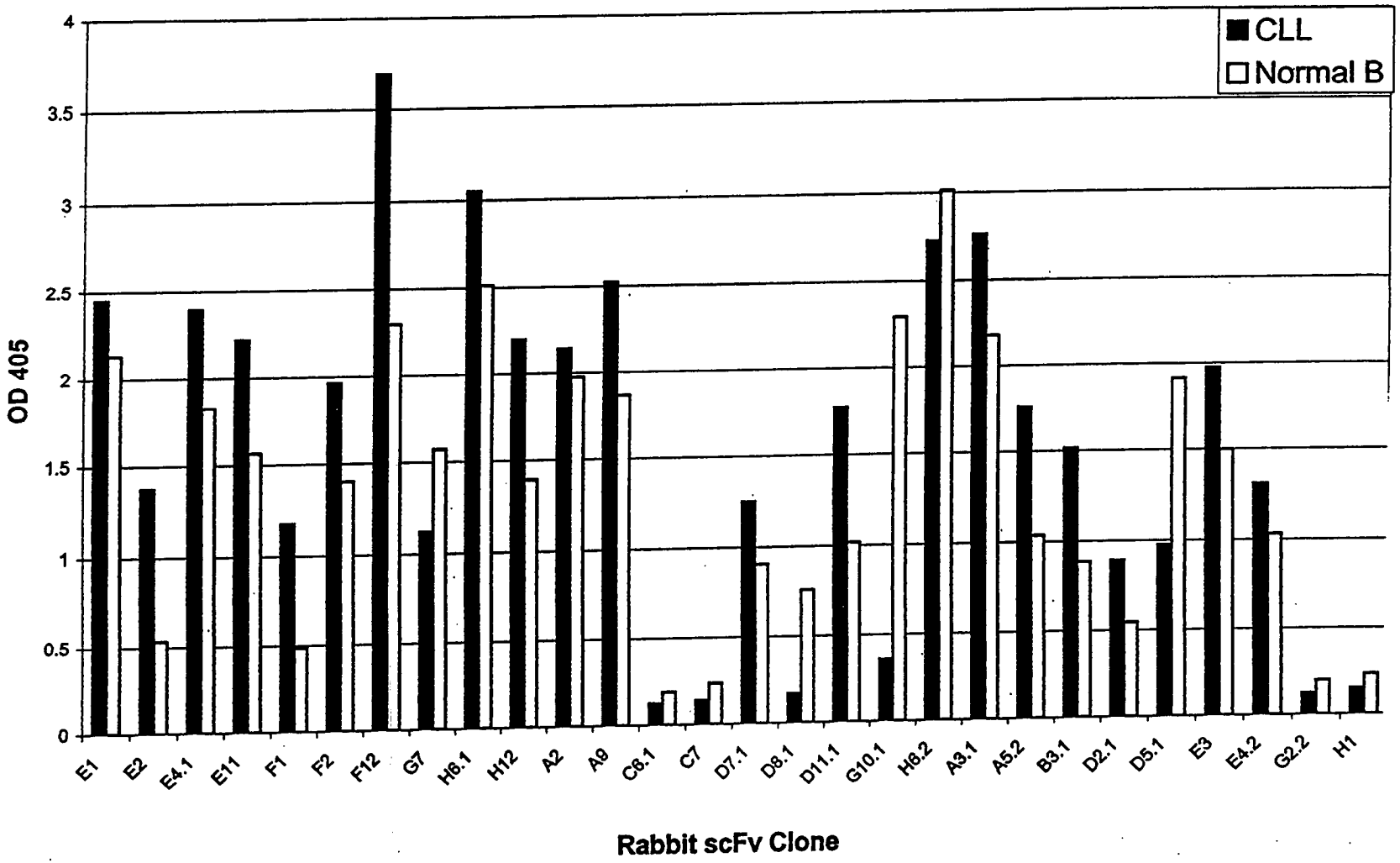

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Fig 3b. CELL ELISA 4/22/00

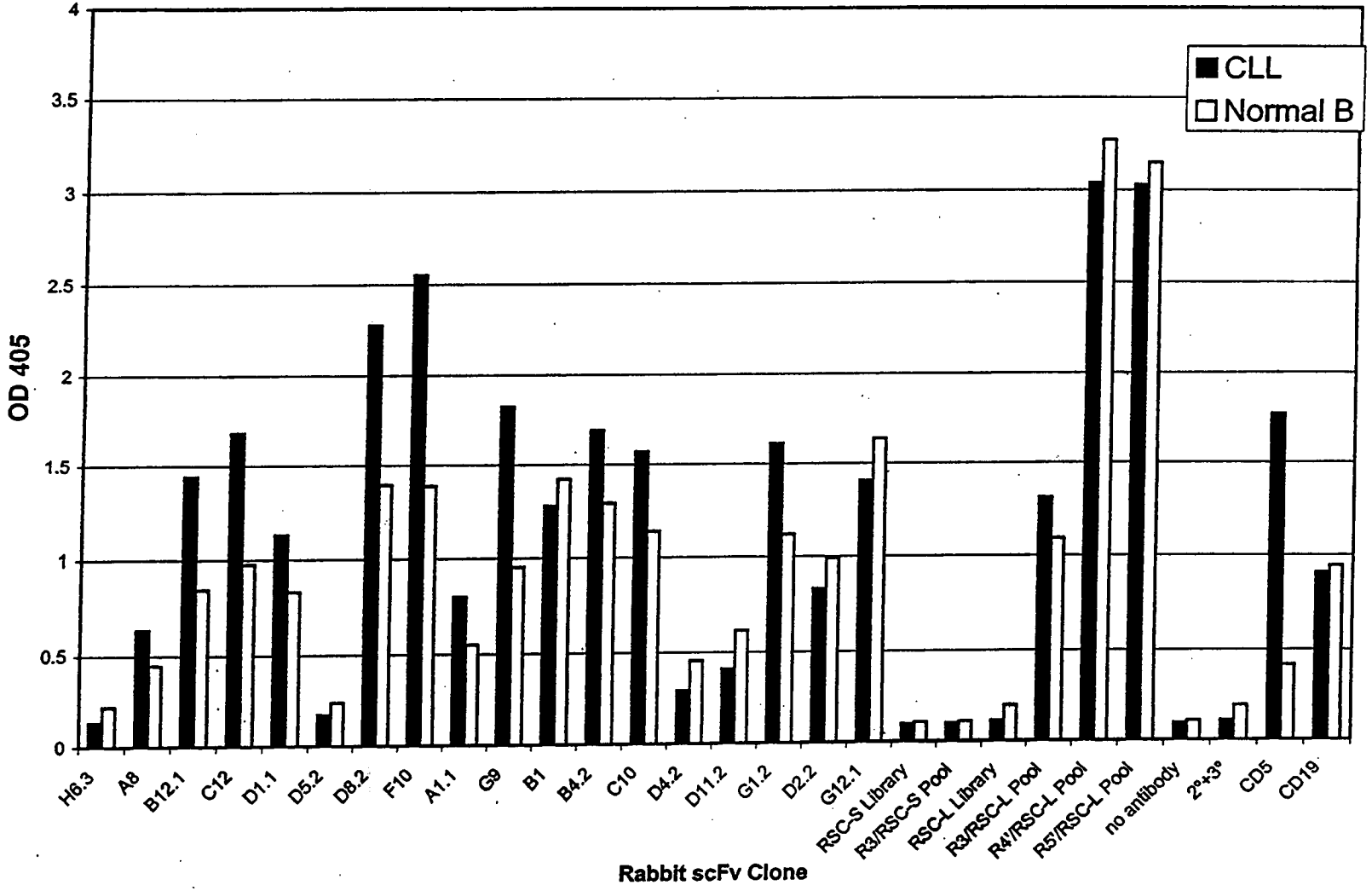

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Fig 4a. CELL ELISA 5/21/00

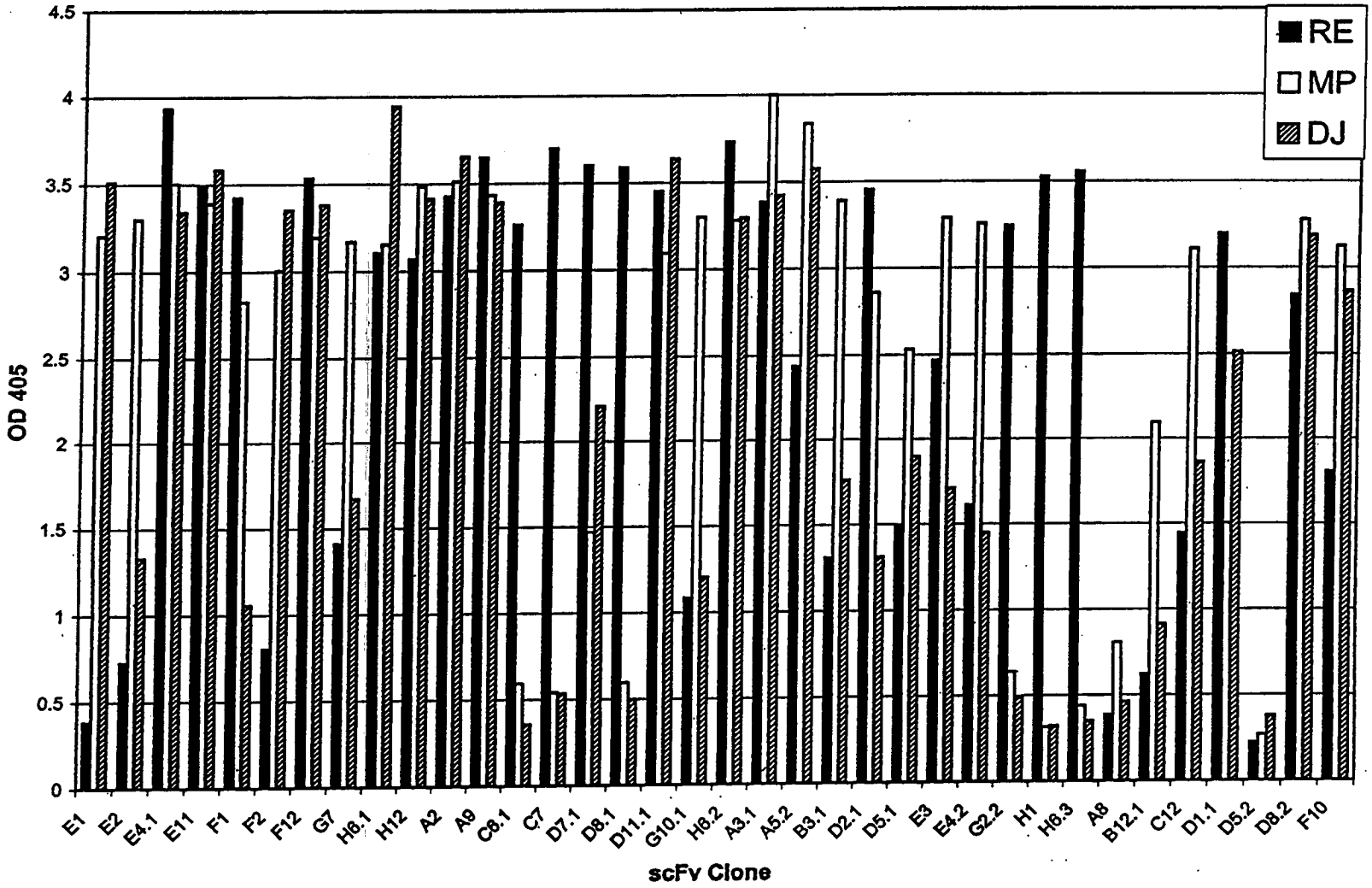

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Fig 4b. CELL ELISA 5/21/00

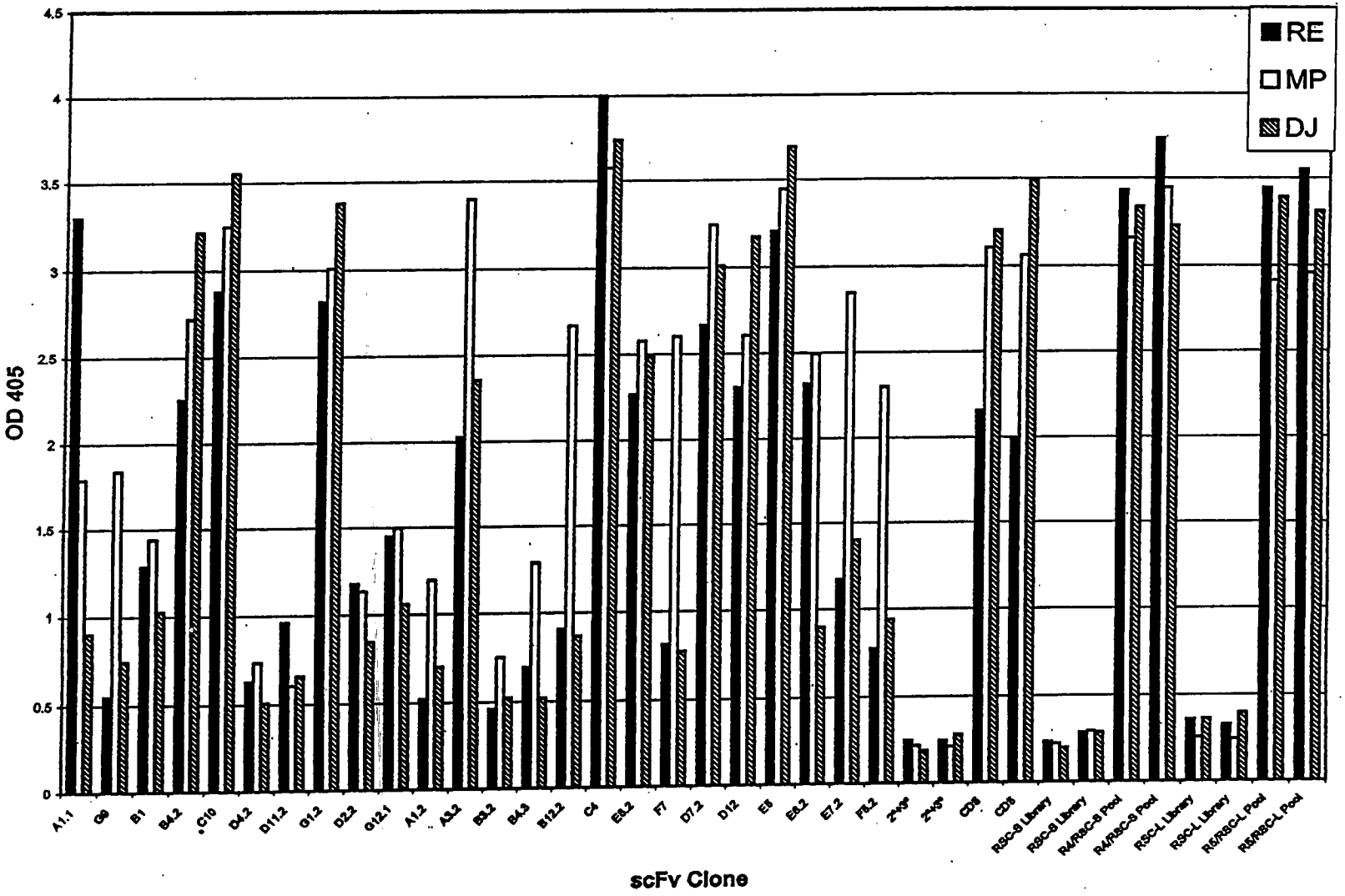

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Fig 5a. CELL ELISA 8/19/00

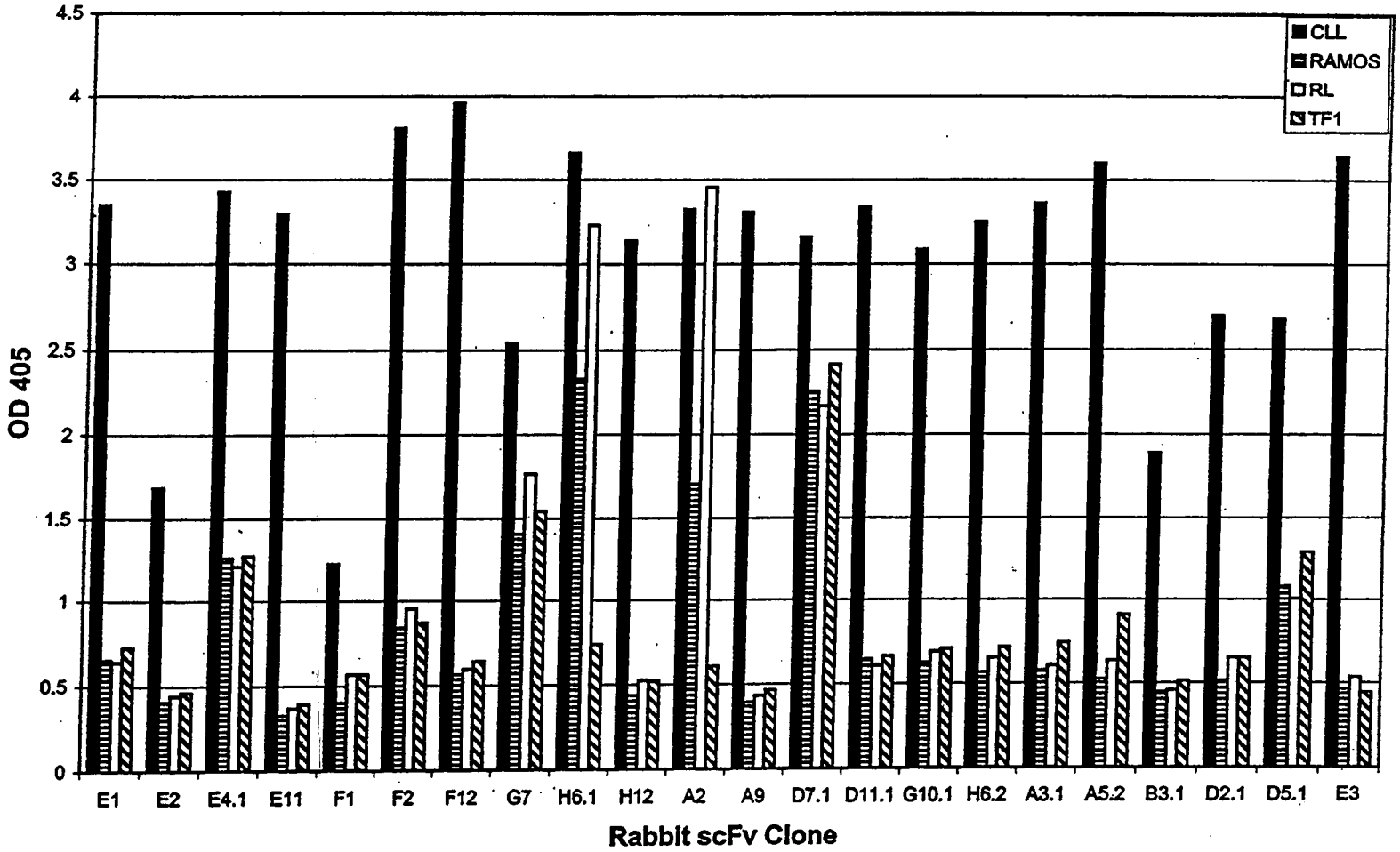

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Fig 5b. CELL ELISA 8/19/00

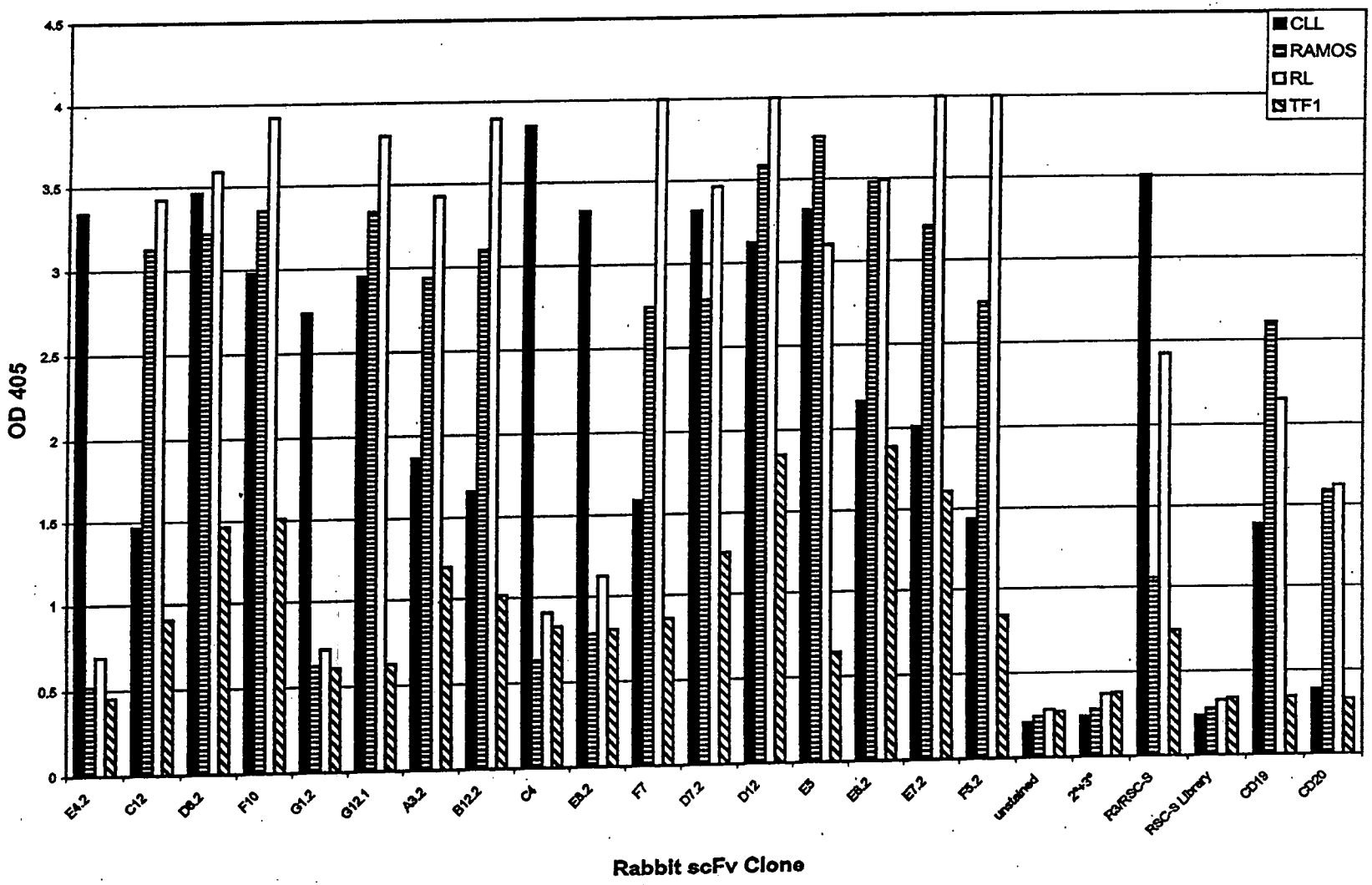

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Fig 6a. CELL ELISA 9/15/00

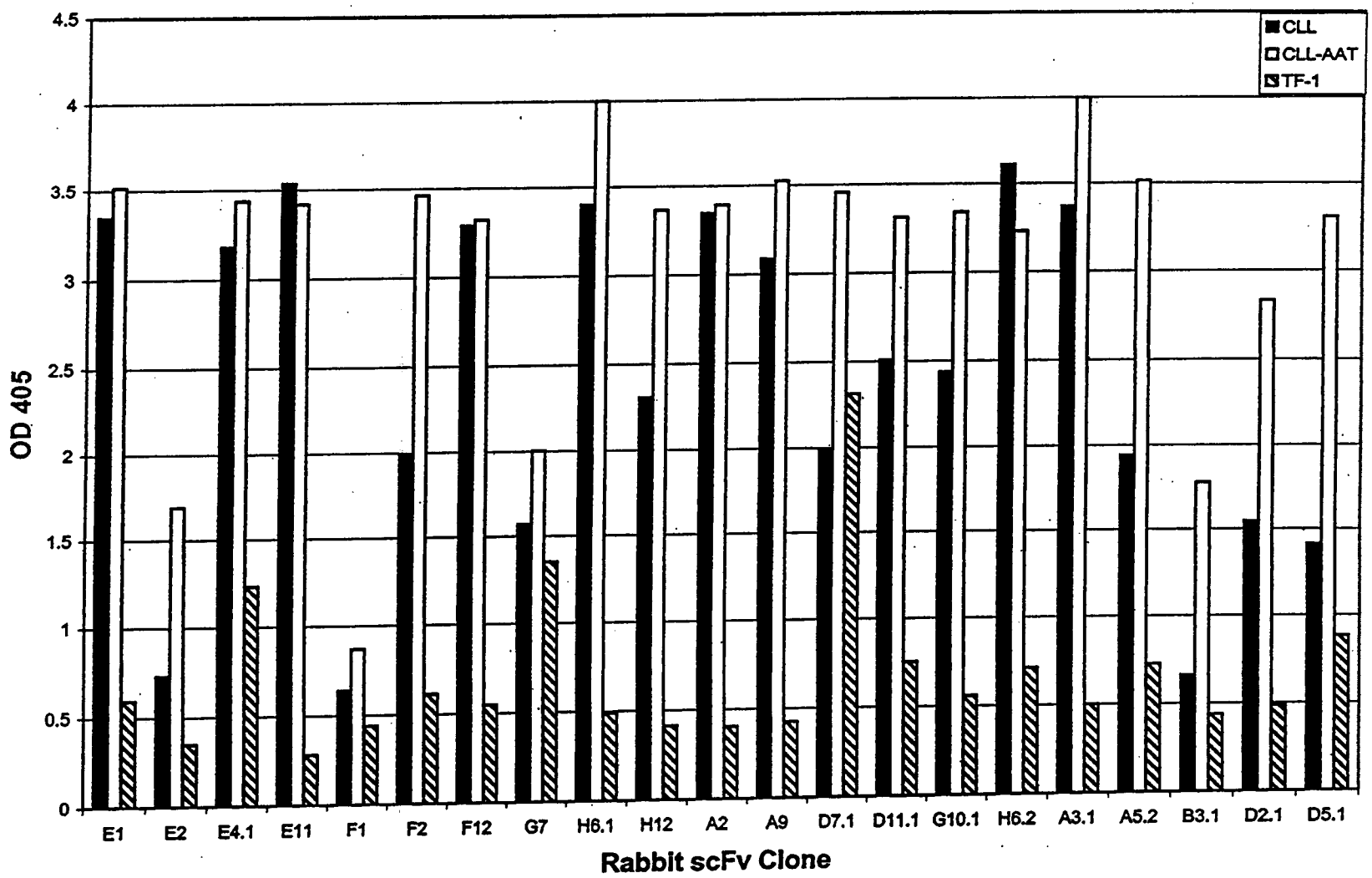

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Fig 6b. CELL ELISA 9/15/00

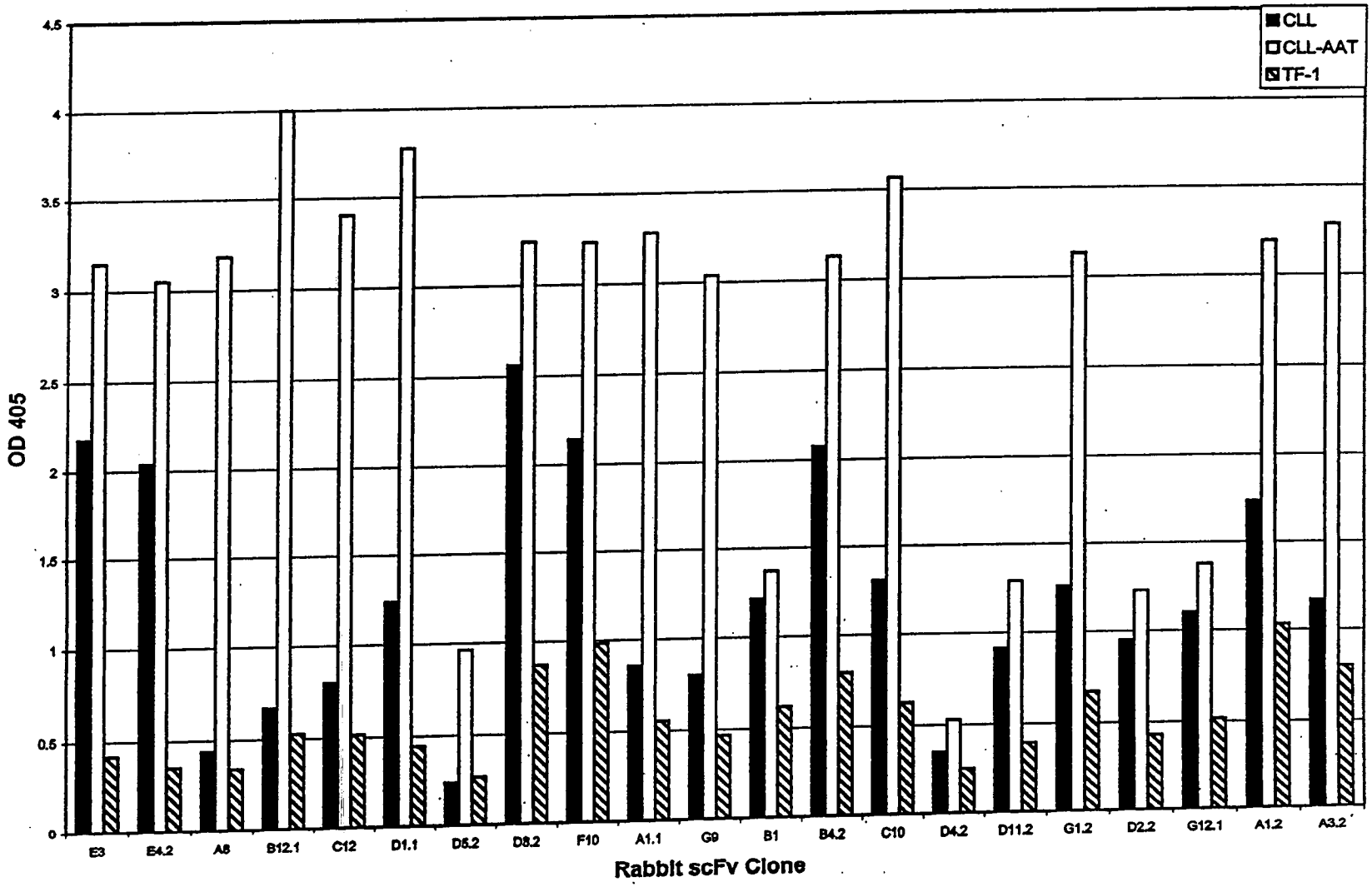

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Fig 6c. CELL ELISA 9/15/00

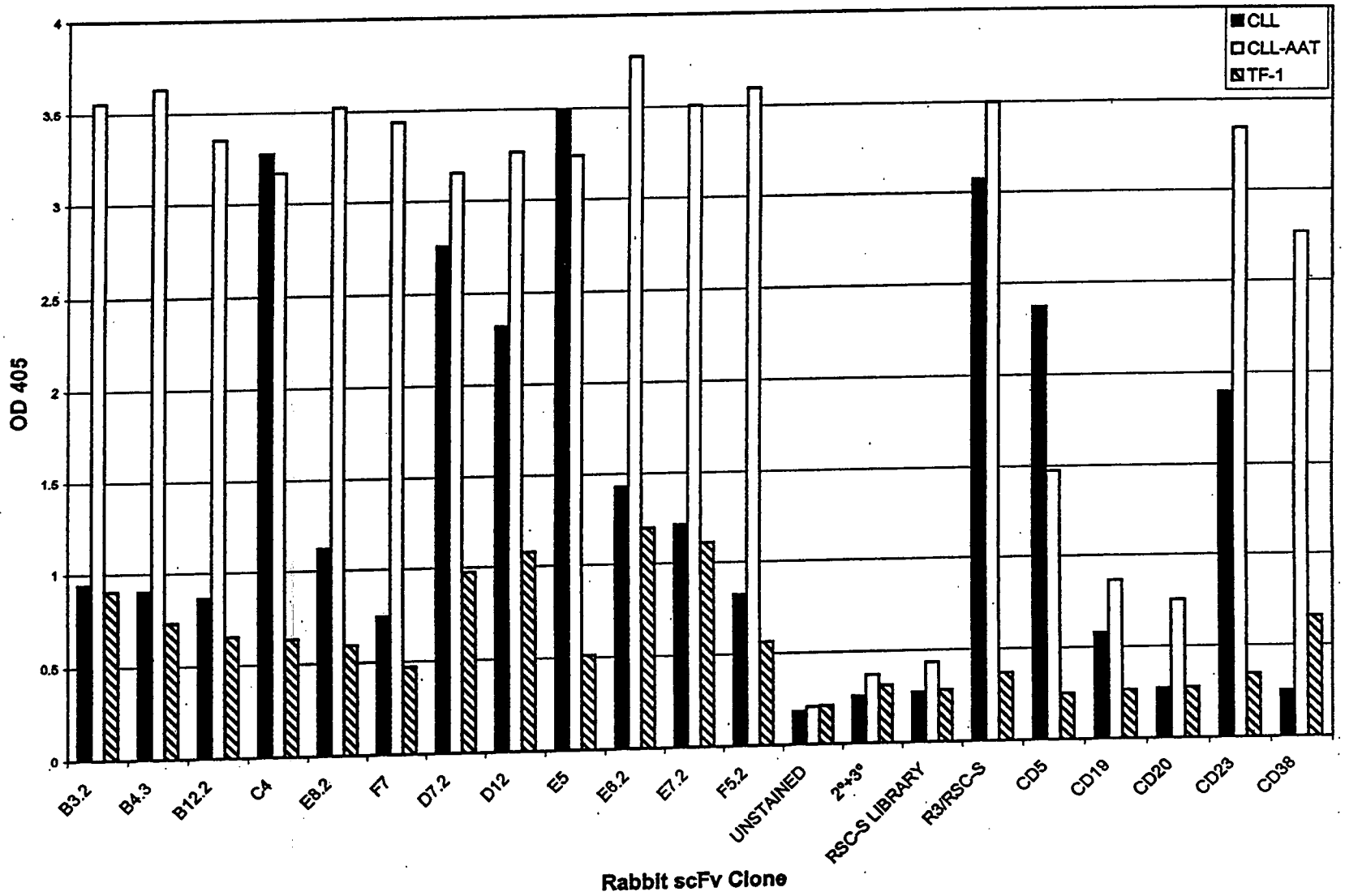

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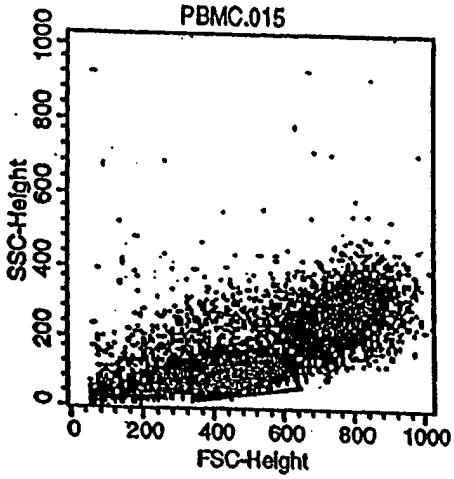
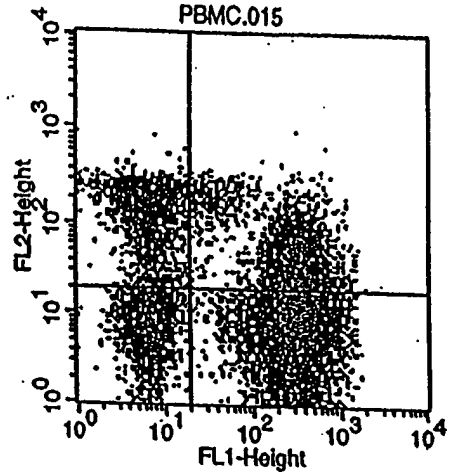
Figure 7

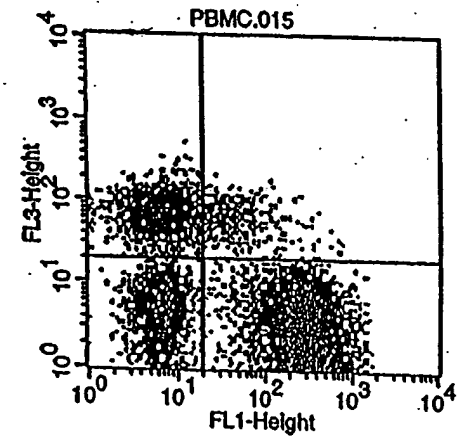
FL2: scFv-9/HA-biotin/SA-PE
FL1: CD5-FITC
FL3: CD19-PerCP





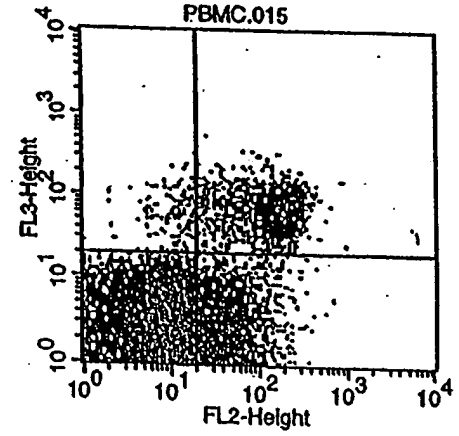
File: PBMC.015
X Parameter: FL1-H FL1-Height (Log)
Y Parameter: FL2-H FL2-Height (Log)

Quad	Events	% Gated	% Total	X Geo Mean	Y Geo Mean
UL	1881	9.40	5.84	6.45	118.74
UR	4368	21.84	13.56	266.89	45.49
LL	2831	14.16	8.79	6.65	7.40
LR	10920	54.60	33.90	282.52	5.72



File: PBMC.015
X Parameter: FL1-H FL1-Height (Log)
Y Parameter: FL3-H FL3-Height (Log)

Quad	Events	% Gated	% Total	X Geo Mean	Y Geo Mean
UL	1874	9.37	5.82	6.55	65.56
UR	409	2.04	1.27	50.57	55.81
LL	2838	14.19	8.81	6.57	4.19
LR	14879	74.39	46.19	291.30	2.17



File: PBMC.015
X Parameter: FL2-H FL2-Height (Log)
Y Parameter: FL3-H FL3-Height (Log)

Quad	Events	% Gated	% Total	X Geo Mean	Y Geo Mean
UL	171	0.85	0.53	10.16	54.88
UR	2112	10.56	6.56	137.20	64.47
LL	13744	68.72	42.67	6.08	2.52
LR	3973	19.86	12.33	41.31	2.08

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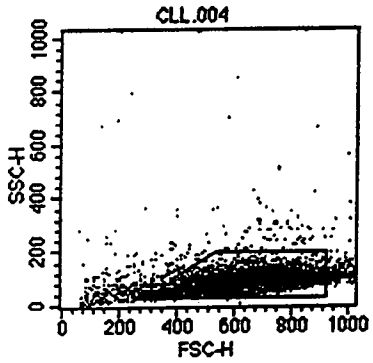
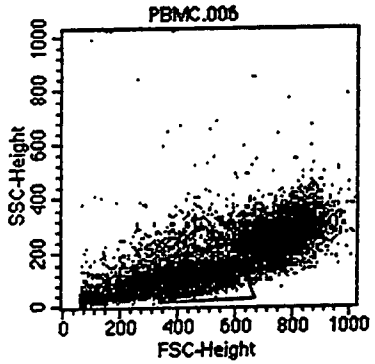
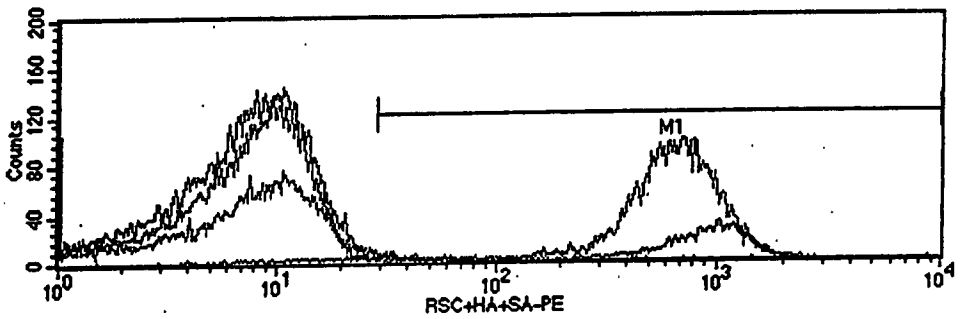
Figure 8a





scFv-2



Key	Name	Parameter	Gate
—	CLL.004	FL2-H	G3
—	CLL.006	FL2-H	G3
—	PBMC.006	FL2-H	G1
—	PBMC.008	FL2-H	G1

* Numerator Histogram *
File: CLL.006

* Denominator Histogram *
File: CLL.004

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 991	10233	99.57	86.52	654.78	553.65	47.14	620.82	685
M1	28, 991	10028	97.58	84.79	667.84	598.59	44.59	626.43	685

* Numerator Histogram *
File: PBMC.008

* Denominator Histogram *
File: PBMC.006

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 991	20309	99.96	64.48	111.91	12.05	280.93	8.98	1
M1	28, 991	2403	11.83	7.63	881.67	708.77	45.81	897.69	1000

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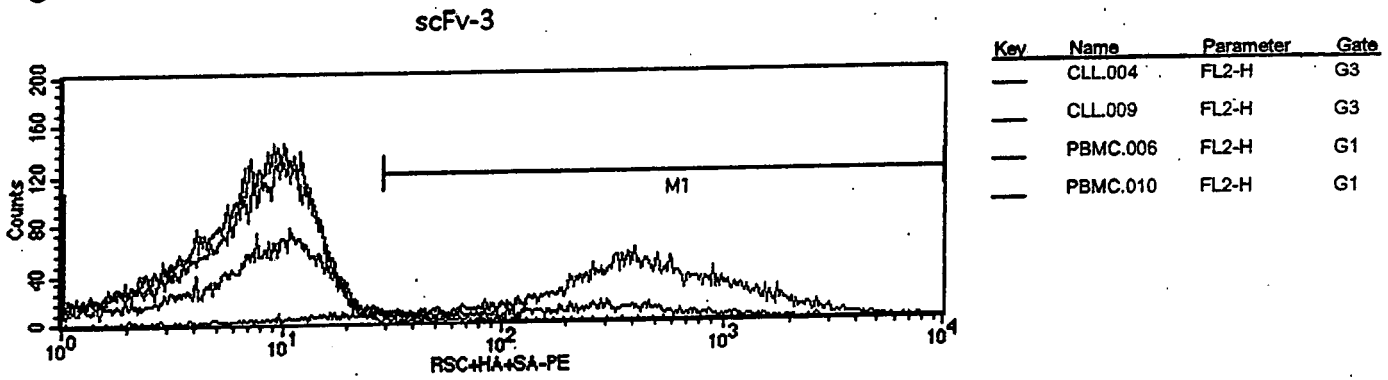
Figure 8b



* Numerator Histogram *
File: PBMC.010

* Denominator Histogram *
File: PBMC.006

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 991(	20389	100.12	64.95	34.40	8.94	351.39	8.35	1
M1	28, 991(	1953	9.59	6.22	281.98	176.73	103.08	201.69	271

* Numerator Histogram *
File: CLL.009

* Denominator Histogram *
File: CLL.004

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 991(	10258	99.82	86.73	587.42	345.59	104.91	392.42	385
M1	28, 991(	9788	95.24	82.76	614.87	403.58	100.46	414.18	385

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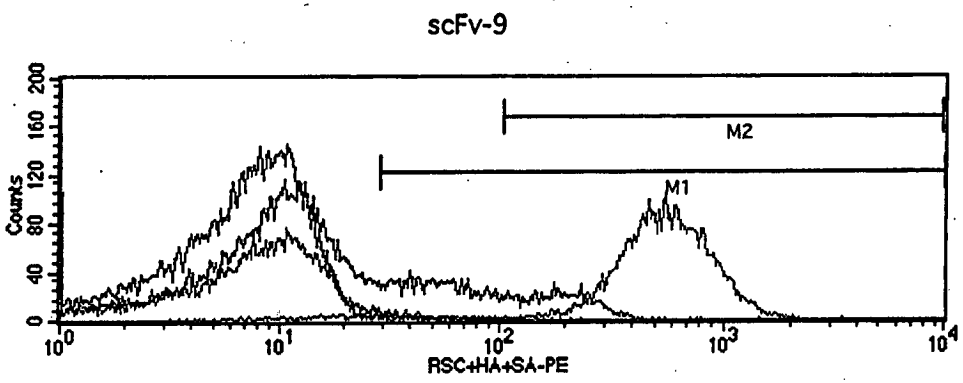
Figure 8c



Key	Name	Parameter	Gate
—	CLL.004	FL2-H	G3
—	CLL.018	FL2-H	G3
—	PBMC.006	FL2-H	G1
—	PBMC.014	FL2-H	G1

* Numerator Histogram *
File: CLL.018

* Denominator Histogram *
File: CLL.004

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 991(	10272	99.95	86.85	568.1(	481.42	48.32	526.02	528
M1	28, 991(	10073	98.01	85.17	579.0(	515.6(	45.93	532.8(	528
M2	103, 991(	9924	96.57	83.91	587.0(	534.83	44.25	537.61	528

* Numerator Histogram *
File: PBMC.014

* Denominator Histogram *
File: PBMC.006

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 991(	20356	100.1(	64.63	34.36	15.09	173.4(	12.30	1
M1	28, 991(	5229	25.74	16.6(	103.57	80.28	82.38	71.05	38
M2	103, 991(	1893	9.32	6.01	190.4(	179.03	45.79	177.83	168

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Figure 9a

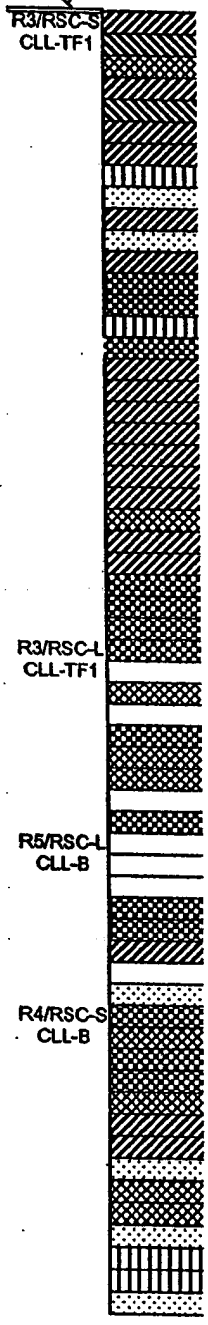

Table 1. Summary of CLL scFv Clones

Clone	CLL	Primary B	CLL-AAT	RL (NHL)	Ramos (Burkitt's)	TF1	Patient-Specific	Expression Lost	Fingerprint
E1	++	++	++	-	-	-			1
E2	+	-	+	-	-	-			2
E4.1	++	+	++	±	±	±			3
E11	++	+	++	-	-	-			4
F1	+	-	+	-	-	-			5
F2	++	+	++	-	-	-			6
F12	++	+	++	-	-	-			7
G7	+	+	+	+	+	+			8
H6.1	++	+	++	+	+	-			7
H12	++	+	++	-	-	-			9
A2	++	++	++	+	+	-			10
A9	++	+	++	-	-	-			11
C6.1	-	-	nd	nd	nd	nd	+		12
C7	-	-	nd	nd	nd	nd	+		13
D7.1	+	+	+	+	+	+			14
D8.1	-	+	nd	nd	nd	nd	+?		15
D11.1	++	+	++	-	-	-			16
G10.1	+	+	+	-	-	-			17
H6.2	++	++	++	-	-	-			18
A3.1	++	+	++	-	-	-			19
A5.2	++	+	++	-	-	-			20
B3.1	+	+	+	-	-	-			9
D2.1	+	+	+	-	-	-			21
D5.1	+	+	+	±	±	±			22
E3	+	+	+	-	-	-			23
E4.2	+	+	+	-	-	-			24
G2.2	-	-	nd	nd	nd	nd	+		25
H1	-	-	nd	nd	nd	nd	+		26
H6.3	-	-	nd	nd	nd	nd	+		27
A8	-	+	+	nd	nd	nd	+?		28
B12.1	+	+	++	nd	nd	nd			29
C12	++	+	++	+	+	±			30
D1.1	+	+	+	nd	nd	nd			31
D5.2	-	-	+	nd	nd	nd	+?	+	32
D8.2	++	+	++	+	+	±			33
F10	++	+	++	+	+	±			34
A1.1	+	+	++	nd	nd	nd			(nd)
G9	±?	+	++	nd	nd	nd	+?		(nd)
B1	+	+	+	nd	nd	nd			35
B4.2	++	+	++	nd	nd	nd			36
C10	++	+	++	nd	nd	nd			37
D4.2	-	-	-	nd	nd	nd	+?	+	38
D11.2	±?	-	+	nd	nd	nd	+?		39
G1.2	++	+	++	-	-	-			37
D2.2	+	+	+	nd	nd	nd			40
G12.1	+	+	+	+	+	-			41
A1.2	±?	nd	++	nd	nd	nd	+?		42
A3.2	+	nd	++	+	+	±			43
B3.2	-	nd	+	nd	nd	nd	+		44
B4.3	-	nd	++	nd	nd	nd	+		45
B12.2	+	nd	++	+	+	±			46
C4	++	nd	++	-	-	-			47
E8.2	++	nd	++	-	-	-			48
F7	+	nd	++	+	+	-			46
D7.2	++	nd	++	+	+	±			49
D12	++	nd	++	+	+	+			50
E5	++	nd	++	+	+	-			51
E6.2	+	nd	++	+	+	+			52
E7.2	+	nd	++	+	+	+			53
F5.2	+	nd	++	+	+	-			54

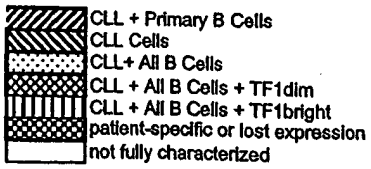

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FIGURE 9B

Table 1. CDR Sequences of CLL Specific Rabbit scFv Antibodies

SEQ	CLONE	LC-CDR1	LC-CDR2	LC-CDR3	HC-CDR1	HC-CDR2	HC-CDR3	Expression Pattern: CLL	B	RL	Ramos	TF-1	Ag	Linker
1	A2c	TLSTGYSVGSYVIA	HSEEAKHQGS	ATAHGSGSSFHVV	NYAMT	IISSNGGA--DYASWAK	DDEGYDDYGDYMGYFTL	+	+	++	+	-		S
2	G12.1c	QASESIRN---YLA	GASNL---ES	QSGDYSA---GLT	SYGLS	YFDPVFGNT-YYATWVD	DRIYVSSVG---YAFNL	+	+	+	+	-	CD19	L
17	B4.2a	QASESIRN---YLA	GASNL---ES	QSGYYSA---GLT	TYGVS	YNDPIFGNT-YYATWVN	DRAYASSSG---YCCCX	+	nd	+	+	-		L
3	E1c	QASESISN---WLA	RASTL---AS	QSGYYSA---GVT	SNAMG	IISSSGGT--YYASWAK	DWIAAGKS----YGLDL	++	+	-	-	-	CD23	S
18	F2d	QASESISN---YLA	GASNL---ES	QSGYYSA---GLT	TNAMG	IISSSGST--YYASWAK	DWIAAGKS----YGLDL	++	+	-	-	-		S
4	E5e	QASQNIYS---NLA	LAFTL---AS	QGGDYSSSSSYGYG	SSDWIC	CIYTGSSSSTWYASWAK	RYTGDNG--------NL	±	nd	-	-	-		S
5	H6.2b	QASQSVNN---LLA	GASNL---ES	QSGYYSP---GVT	SDVIS	YIYTGDGST-DYASWVN	DAAYAGYGW----IFNL	++	++	-	-	-		S
19	G10.1	QASESINN---YLA	GASNL---ES	QSGYYSG---GAT	SDVIS	YIYTGDGST-DYASWVN	DAAYAGYGW----IFNL	+	+	-	-	-		S
6	D11.1c	LASENVYS---AVA	GASDL---ES	Q-GYSSYPPT	TYAMG	SIYASRSP--YYASWAK	GDAGSIP------YFKL	++	+	-	-	-	CD23	S
20	A5.2c	LASENVYG---AVA	GASNL---ES	Q-GYSSYP-T	TYAMG	SIYASRSP--YYASWAK	GDAGSIP------YFKL	++	+	-	-	-		S
7	F1d	QASQSVNN---LLA	GASNL---ES	AGYKSSSTD-GIA	SNAMT	TIIYGDNT--YYASWAK	GNV----------FSDL	+	±	-	-	-		S
8	F1e	QASQSISN---LLA	GASNL---ES	QSGYYSA--GHLT	DFAMS	VVYAGTRGDTYYANWAK	GLT----------YYPL	++	nd	-	-	-		S
21	E4.2	LASENVAS---TVS	GASNL---ES	LGGPGYSTT-GLT	DFAMS	VVYAGTRGDTYYANWAK	GLT----------YYPL	+	+	-	-	-		S
9	E2c	TLSTGYSVGEYPVV	HTDDIKHQGS	AIAHGTESSFHVV	SYGMN	YIDPDYGST-YYASWVN	GAYSGYPS-----YFNL	+	±	-	-	-		S
	A9c	TLSTGYSVGEYPVV	HTDDIKHQGS	AIAHGTESSFHVV	SYGMN	YIDPDYGST-YYASWVN	GAYSGYPS-----YFNL	++	+	-	-	-		S
10	E11e	TLRTGYSVGEYPLV	HTDDIKHQGS	ATGHGSGSSAGVV	SNAMS	ITYPSGNV--YYASWAK	G------------FFNL	++	+	-	-	-		S
11	A1.1	LASEDIYS---GLS	GASNL---ES	LGGYPYSST-GTA	TNAIS	YSSYGNNA--HYTNWAK	GNA----------YSDL	+	+	nd	nd	nd		L
12	F5.2	QASQSVSN---LLA	GASNL---ES	QSGWYSA--GALT	SNAMS	IIIGSGTT--YYANWAK	DQPIIYGAYGDYGLATGTRLDL	+	nd	+	+	-		L
22	F10b	QASQSVNN---LLA	RASTL---AS	QSGYYRA--GDLT	SYIMS	IISSSGIS--YYATWAK	DQPIIDAAYGDYGIATGTRLDL	nd	nd	nd	nd	nd		L
23	F7a	QASQSVSN---LLA	GASNL---ES	QSGYYSA---GLT	SYIMS	IISSSGSA--YYATWAK	DQPIITTDYGGYGIATGTRLDL	nd	nd	nd	nd	nd		L
13	F6b	QASQSVSN---LLA	GASNL---ES	QSGYYSA---GLT	SNAIS	IIVGSGTT--YYADWAK	DQPITYAGYGY---ATGTRLDL	nd	nd	nd	nd	nd		L
24	C12b	QASQSVSN---LLA	GASNL---ES	QSGYYSA--GALT	SNAIS	IIVGSGTT--YYADWAK	DQPITYAGYGY---ATGTRLDL	nd	nd	nd	nd	nd		L
14	D2.1b	QASQSVNN---LLA	GASNL---ES	QSGYYSA---GLT	TNAMS	TITYGTNA--YYASWAK	GNT----------YSDL	nd	nd	nd	nd	nd		S
25	D1.1	QASEDIES---YLA	GASNL---ES	QSNAWSV---GMT	SNAMS	TITYGTNA--YYASWAK	GNT----------YSDL	+	+	nd	nd	nd		L
15	D2.2a	QSSQSIAGA--YLS	LASKL---AS	AAQYSGN---IYT	SSYWIC	CIYTGSNGSTYYASWAK	AYIYYGGYG----FFDL	nd	nd	nd	nd	nd		L
16	D2.2b	LASENVYG---AVA	GASNL---ES	Q-GYSSYP-T	NYGVN	YIDPVFGST-YYASWVN	EASFYY-------GMDL	nd	nd	nd	nd	nd		S

SEQ: sequence designation
CLONE: designation of representative clone for sequence
LC: Ig light chain
HC: Ig heavy chain
CDR: complementarity determining region
Expression pattern: binding of scFv antibodies to primary human cells and cell lines as determined by whole cell ELISA assay
CLL: chronic lymphocytic leukemias (primary tumors and CLL-AAT cell line)
B: normal, primary human B lymphocytes
RL: non-Hodgkin's lymphoma cell line
Ramos: Burkitt's lymphoma cell line
TF-1: human erythroleukemia cell line
Ag: antigen recognized by scFv antibody (determined by immunoprecipitation and mass spectrometry)
Linker: type of linker sequence between VL and VH regions. S, short linker; L, long linker

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FIGURE 10

Table 2. Mean fluorescent intensities of B-CLL cells and normal PBMC labeled with scFv antibodies

	Antibody and CLL/PBMC Ratio:							
Donor	scFv-2	ratio	scFv-3	ratio	scFv-6	ratio	scFv-9	ratio
CLL(ML)	590	0.83	398	2.2	284	2.1	511	6.4
PBMC-1	715		181		137		80	
CLL(JR)	311	0.85	207	2.4	nd	nd	117	1.7
PBMC-2	368		87		nd		67	
CLL(HTS)	219	0.69	173	1.6	nd	nd	176	3.6
PBMC-3	317		106		nd		49	
CLL(RE)	305	0.59	360	3	nd	nd	142	1.7
PBMC-4	513		121		nd		81	
CLL(GB)	262	0.47	387	1.8	nd	nd	163	1.5
PBMC-5	563		212		nd		106	

Primary PBMC from five patients diagnosed with CLL and five normal donors were analyzed by flow cytometry. The geometric mean fluorescent intensities were determined for cells stained with four different scFv antibodies. For scFvs that bind to antigens overexpressed on CLL cells, the CLL/PBMC ratio of fluorescent intensities is >1.0.

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Fig 11. Comparison of scFv-9 antigen and CD38 expression on CLL cells.

Patient ID	% $CD19^+$	% $CD38^+$	% $scFv\text{-}9^+$	ScFv-9 Level	CD38	ScFv-9
ML	80	40	98	266	Hi	Hi
IB	86	87	96	366	Hi	Hi
BH	76	56	86	284	Hi	Hi
JG	82	92	97	125	Hi	Lo
RE	87	97	100	125	Hi	Lo
EM	91	8	95	268	Lo	Hi
HS	76	11	94	268	Lo	Hi
MP	40	6	95	280	Lo	Hi
JR	81	12	92	124	Lo	Lo
GB	65	20	98	187	Lo	Lo

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Fig 12. Identification of scFv Antigens

Cell-surface biotinylation (CLL-AAT cells)

Membrane isolation (nitrogen cavitation, differential centrifugation)

Immunoprecipitation with scFv-HA coupled to Anti-HA beads

•SDS-PAGE

Detection by Coomassie-stain or AP-streptavidin Western blot

•MALDI-MS or LC-MS/MS to obtain peptide mass spectra/peptide sequences

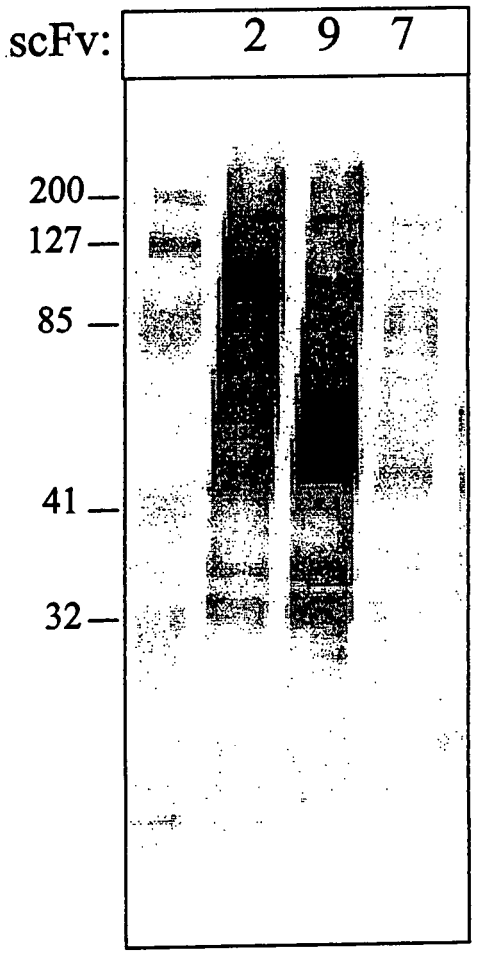

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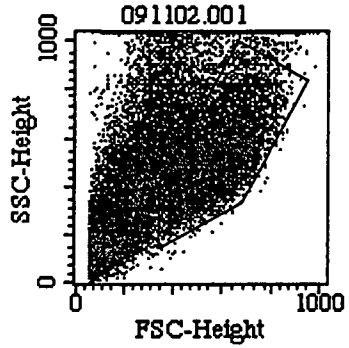
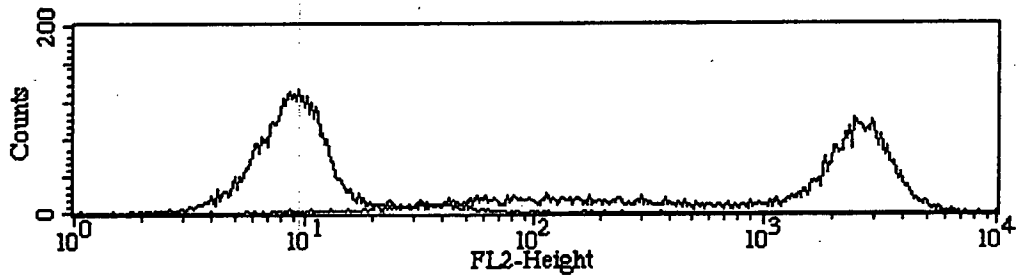
Figure 13

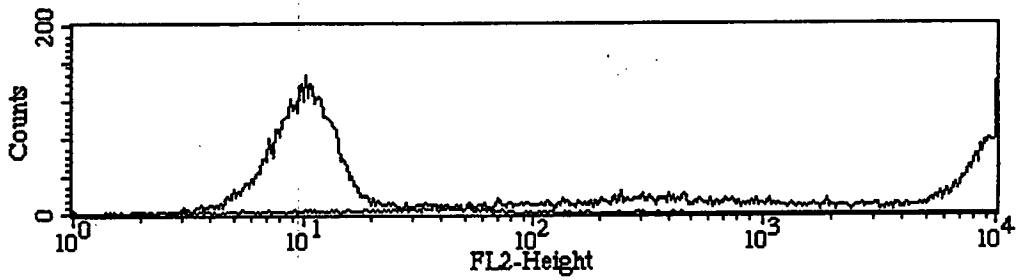


Key	Name	Parameter	Gat
293-pCEP4: —	091102.002	FL2-H	G1
293-CD200: —	091102.008	FL2-H	G1

scFv-4



scFv-9



scFv-10

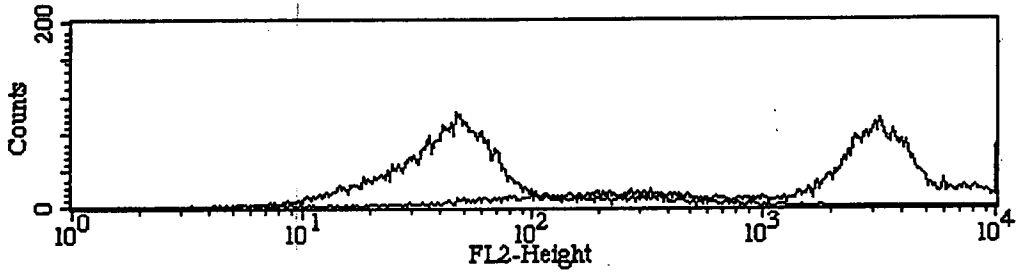


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Figure 14

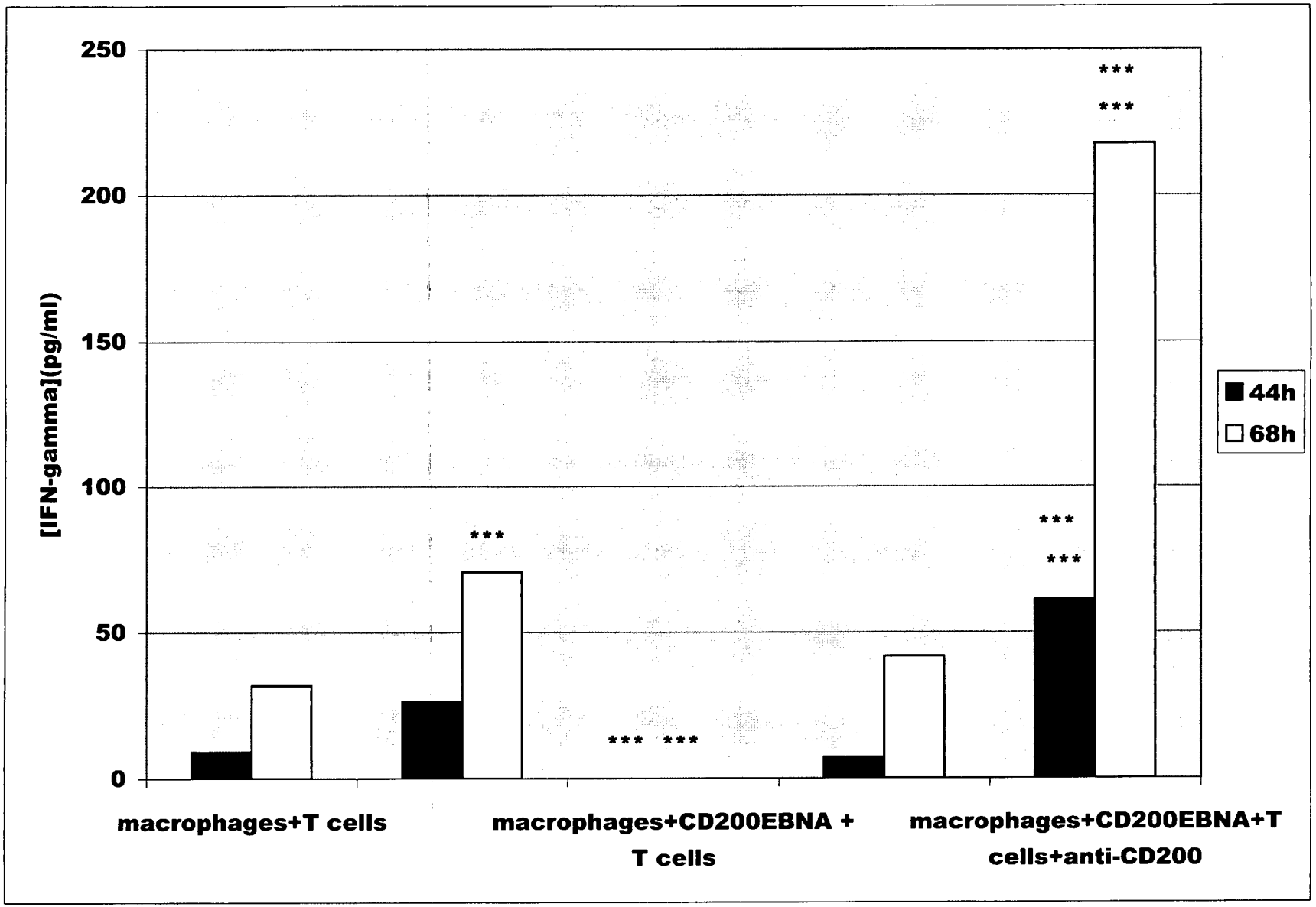

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Figure 15

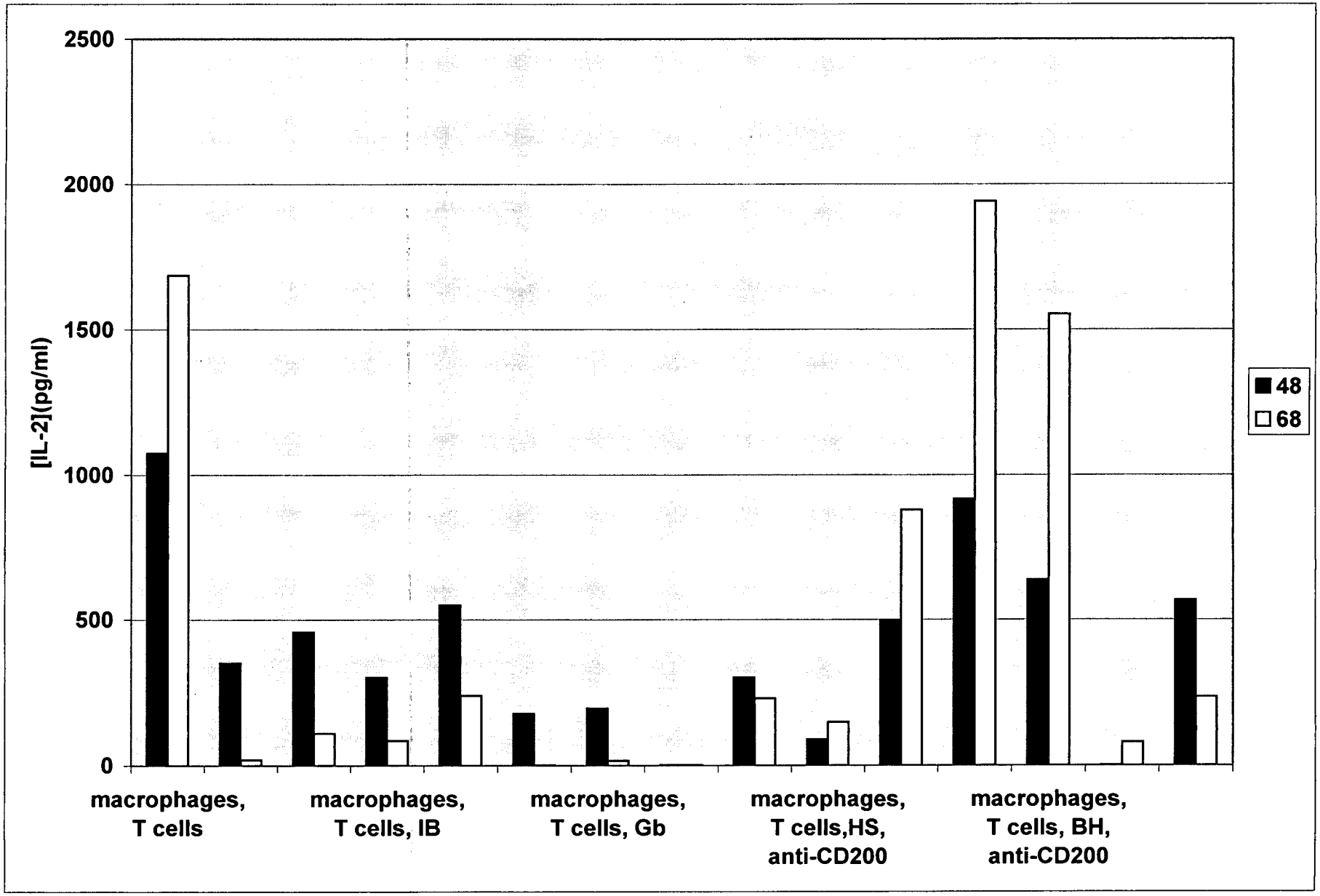
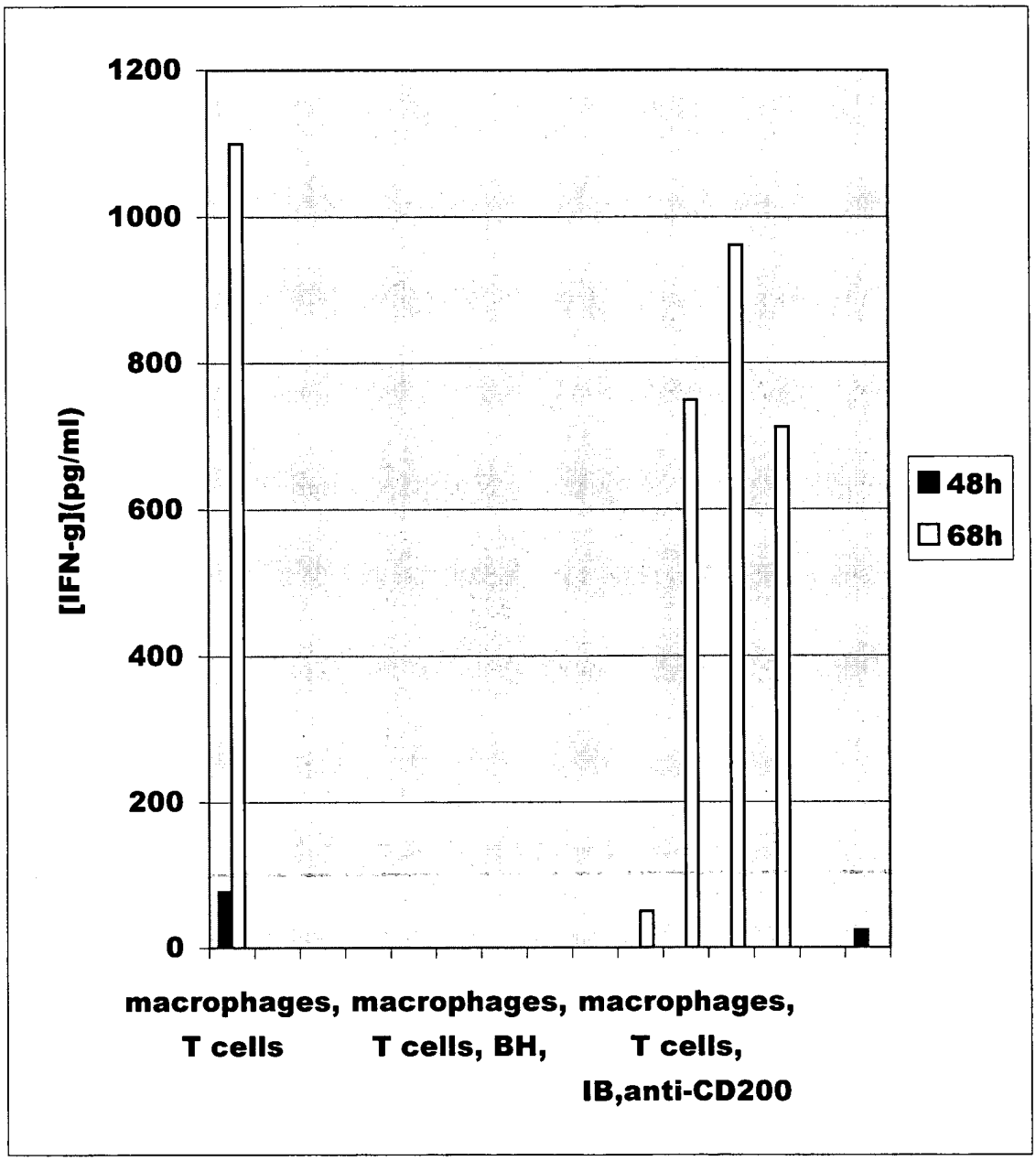


Figure 16



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